

EXHIBIT F



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(54) Title: PHARMACEUTICAL COMPOSITIONS CONTAINING PLASMA PROTEIN		
(57) Abstract <p>The invention is related to water-soluble products and pharmaceutical formulations in solid or liquid form mainly for parenteral use. They consist of or comprise a therapeutically active substance (having low aqueous solubility and a substantial binding affinity to plasma proteins) and a plasma protein fraction in controlled aggregation state, whereby the said active substance and the said protein fraction are bound to each other by way of noncovalent bonds. It also covers processes for the preparation of the product and pharmaceutical formulation.</p>		

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PHARMACEUTICAL COMPOSITIONS CONTAINING PLASMA PROTEIN

The present invention is related to a new method, products and formulations for delivery in therapeutic use of therapeutically active compounds having poor water solubility and substantial binding affinity to plasma proteins and processes for the preparations of such products and formulations.

More particularly first objects of the invention are products and pharmaceutical formulations in solid or liquid form mainly for parenteral use consisting of or comprising

a) a therapeutically active substance having low aqueous solubility and a substantial binding affinity to plasma proteins (in the following "active substance") and

b) a plasma protein fraction in controlled aggregation state

whereby the said active substance and the said protein fraction are bound to each other by way of non-covalent bonds and

c) optionally further pharmaceutically acceptable and mainly parenterally acceptable formulation additive(s) - such as water, stabilizer(s), protein aggregation controller(s).

The homogeneous solid state products of the invention consisting of the said protein and the said substance are water-soluble and their aqueous solutions can be used parenterally or can be used to prepare parenteral pharmaceuticals.

It is well known in the art that some biologically active compounds possess potent therapeutic activity but could never demonstrate their benefit because of their poor solubility in aqueous media. Some of them were never ever formulated while a few did not reach but the stage of the "phase I" clinical development. Some of them appear in "hardly biocompatible" formulations of relatively high toxicity caused by the materials used for formulation. A typical example for this is represented by the groups of taxones specifically paclitaxel which is a potent cytostatic the application of which however is reduced because of the toxicity of its known formulation in Klucel : tween 80 or Klucel and diluent 12, a 1:1 mixture of Cremaphor EL : ethanol. [Cancer Chemotherapy and Pharmacology (1994) 34:465-471; Journal of the National Cancer Institute (1990) 1247-1259]. Cremaphor EL (polyoxyethylated castor oil) has

inherent toxicity, causing vasodilatation, lethargy, hypotension etc. In order to decrease the toxic side-effect of the solvent and adjuvant, a series of special methods were suggested: application of very small doses over a long period of time, pre-medication before treatment etc. (USP 5665761; USP 5621001; USP 5670537 etc.) A further suggestion consisted in combination of the active substance with a dispersing agent contained within a protein walled shell (USP 5 560 933) which is formed by reacting the protein with oil such as soy bean oil - such formulations being proposed for paclitaxel and amphotericin. However even the latest literature comprises warnings on the course of application of e.g. paclitaxel (see e.g. "Guidance for Industry issued by the U. S. Department of Health and Human Service CDER September 1997, OGD-L-8) where - because of hypersensitivity reactions - all patients treated with paclitaxel should be premedicated with corticosteroids, diphenhydramine and H₂ antagonists.

It was further proposed to prepare parenteral formulations of certain water-insoluble dihydropyridins, by dissolving them in an organic solvent or in a mixture of an organic solvent with water and adding an aqueous HSP solution to said solution in order to minimise crystallisation of the insoluble active substance (Hungarian Patent N° 198361; DE Appl. 37 02105). The resulting liquid however was still not a clear solution.

It is further known that some of the water-insoluble active substances possess a considerable affinity to protein or serum protein. Some literature is mentioned here for paclitaxel [Cancer Chem. and Pharm.(1994)34: 465 - 471]; miconazole, fluconazole, amphotericin B [Infection, 23(5): 292 - 297 (1995) Sept.]; carbamazepine [J. Chromatogr. B Biomed. Appl. 669(2): 281 -288 (1995 July 21)]; azathioprine [Ann. N.Y. Acad. Sci, 685 (1993): 175 - 192], propofol [J. Chromatogr. Sci (1992): 164 - 166]. According to new literature [The Lancet vol. 352 (1998): 540-542] the drug Taxol® caused rouleaux formation of red cells and so did polyoxyethylated castor oil which served as the solvent of said drug. Some water-insoluble drugs were formulated using the toxic Cremaphor (cyclosporin, teniposide, paclitaxel, amphotericin B). To the best of our knowledge a series of highly active but water-insoluble drugs was not available so

far on the market in parenteral, intravenous administration forms at all e. g. ritonavir, carbamazepine, camphothecine, azathioprine, miconazole, fluconazole etc.

Thus there is a need to solve the problem whereby therapeutically valuable water-insoluble substances can be administered in water-soluble form, preferably parenterally to a patient in need to be treated with said active ingredients.

The aim of this invention is to meet this requirement concerning practically water-insoluble active ingredients having a substantial binding affinity to plasma proteins.

The present invention is based on the recognition that binding the active substances to adequate proteins with non-covalent bonds before administration presents a new and highly potential delivery system for the administration of the active ingredients with poor water solubility. According to the invention homogeneous solid products are produced which are then dissolved in water whereby biocompatible, clear, aqueous solutions are obtained which are suitable for parenteral administration. Thus the invention presents a means to administer the desired water-insoluble active ingredients without introducing the toxic elements and in certain cases in a considerable more efficient dose than before.

Definitions used throughout this application which are henceforth not repeated:

R¹ represents tert. butyl-oxy-carboxylic acid amide or benzoyl amide;

R² represents hydrogen or any acyl group preferably acetyl;

Low water-solubility means that the solubility in water at room temperature $< 1.10^{-4}$ M;

Substantial binding affinity to plasma proteins means that >90% of the substance is bound to the proteins in aqueous medium in spontaneous equilibrium at room temperature;

HSA human serum albumin,

WFI water for injection.

One object of the invention is a water-soluble human pharmaceutical formulation mainly for parenteral use containing a therapeutically active compound having low aqueous solubility and a substantial binding affinity to
5 plasma proteins or a human plasma protein fraction in controlled aggregation state.

Other objects of the invention are water-soluble veterinary pharmaceutical formulations mainly for parenteral use containing a therapeutically active compound having low
10 aqueous solubility and a substantial binding affinity to animal plasma proteins in controlled aggregation state.

The human or animal plasmae which can be present in the products and pharmaceutical formulations according to the invention and accordingly used in the methods to prepare the
15 products and compositions can be any of the naturally occurring proteins or plasma fractions such as serum albumin, an immunoglobulin, glycoprotein, interferon and/or interleukin as well as the recombinant analogues of the same. Human and animal proteins can be used. In compounds and
20 compositions intended for treatment of humans the natural human serum and the recombinant human serum proteins are preferred.

The practically water-insoluble active ingredients according to the invention comprise a wide range of compounds
25 whereby the only limitation is that they have to show a substantial affinity to the plasma protein which is selected to be used. Examples for such active ingredients include the following groups of therapeutic agents: a cytostatic such as a taxonoid, antibiotic, vitamin, antiinflammatory,
30 analgesic, anticonvulsant, immunosuppressant, antiepileptic, anxiolytic, hypnotic, antifungal agent, anticoagulant, lipid peroxidase inhibitor, coronary vasodilator, antiarrhythmic agent, cardiotonic, uricosuric, antithrombotic, steroid hormone (progestogen, androgen, testogen) and/or photo-
35 sensitizer. Several active ingredients can be used at the same time after careful consideration and adaptation of the therapeutic doses and consideration of the binding affinities to the selected proteins which have to be able to meet such changed requirements.

40 According to an embodiment of the invention there are provided products and pharmaceutical formulations according

to the above containing at least one of the following active substances: amphotericin B, an adriamycin analogue, apazone, azathioprine, bromazepam, camptothecin, carbamazepine, clonazepam, cyclosporine A, diazepam, dicumarol, digitoxine, dipyridamole, disopyramide, flunitrazepam, gemfibrozil, ketochlorin, ketoconazole, miconazole, niflumic acid, oxazepam, phenobarbital, phenytoin, progesterone, propofol, ritonavir, sulfinpyrazone, suprofen, tacrolimus, tamoxifen, taxonoid, testosterone, tirilazad, trioxsalen, valproic acid and/or warfarin.

A preferred embodiment of the invention consists in a product or formulation as described above containing a taxonoid of the general formula I.

Another preferred embodiment according to the invention contains or consists of paclitaxel and human serum albumin, immunoglobulin, glycoprotein, interferon and/or interleukin or some other human plasma protein fraction.

Further specially important representatives of the invention are homogeneous, solid, water-soluble products consisting of at least one active substance of the group amphotericin B, an adriamycin analogue, apazone, azathioprine, bromazepam, camptothecin, carbamazepine, clonazepam, cyclosporine A, diazepam, dicumarol, digitoxine, dipyridamole, disopyramide, flunitrazepam, gemfibrozil, ketochlorin, ketoconazole, miconazole, niflumic acid, oxazepam, phenobarbital, phenytoin, progesterone, propofol, ritonavir, sulfinpyrazone, suprofen, tacrolimus, tamoxifen, taxonoid, testosterone, tirilazad, trioxsalen, valproic acid and/or warfarin and also consisting of at least one protein of the group human serum albumin, immunoglobulin, glycoprotein, interferon and/or interleukin or some other natural or recombinant human plasma protein fraction where the said active substance and the said protein fraction are bound to each other by way of non-covalent bonds and wherein the molar ratio of the said active substance and the said protein fraction is within the range of 1 : 0.05 to 1 : 100, preferably of 1 : 0.1 to 1 : 50.

Preferred representatives of the above are the following homogeneous, solid, water-soluble products consisting of the following pairs of active substances and proteins:

taxonoid of the general formula I - in the formula

R¹ represents tert. butyl-oxy-carboxylic acid amide or

- benzoyl amide,
R² represents hydrogen or any acyl group preferably
acetyl -
and a plasma protein fraction;
- 5 paclitaxel and human serum albumin, recombinant human plasma
albumin and/or γ -globulin;
amphotericin B and human serum albumin, recombinant human
plasma albumin and/or γ -globulin;
camptothecin and human serum albumin, recombinant human plasma
10 albumin and/or γ -globulin;
carbamazepin and human serum albumin, recombinant human plasma
albumin and/or γ -globulin,
cyclosporin A and human serum albumin, recombinant human plasma
albumin and/or γ -globulin;
15 propofol and human serum albumin, recombinant human plasma
albumin and/or γ -globulin.

It is clear from the above explanations that the
invention covers the pharmaceutical formulations as above
both in the solid state and also in the form of the aqueous
20 solutions.

As it is related to their natural structure - more
specifically to their chemical composition - the protein
molecules tend to aggregate through their specific binding
sites. The degree of aggregation depends on the parameters
25 (temperature, composition, relative and absolute
concentration of the components, consequently the pH, ion
strength) of the solution where the protein is present.

The plasma proteins used according to the invention are
preferably in a stabilized or controlled aggregation state.
30 The aim is to avoid such aggregation of the proteins which
would inhibit optimal binding of the active ingredient
actually used. The unwanted aggregation of the proteins can
be controlled by the presence of other molecules capable to
occupy some or all of the binding sites on the macromolecules
35 involved in the aggregation so as to avoid multiple protein -
protein association. Some proteins are available on the
market in a controlled aggregation state: containing
stabilisers to avoid aggregation. This state however is not
always the optimal state for entering into binding with the
40 active substance we intend to use according to the invention.

According to the invention the term "controlled aggregation state" represents the best binding state when the protein is capable to bind the active substance exactly in the manner which is desired for the purpose aimed at. It is not necessarily the state when the maximum number of the active substance molecules are bound to the protein - but there are cases when the highest binding proportion is desirable.

That means that in some cases we have to remove other excipients from e.g. a commercially available serum albumin fraction, such as stabilisers, ionic components, etc. This might be the necessary starting step of the process when the method according to the invention is carried out. The required conditions to establish the proper aggregation stage strictly depends on the actual active substance and the relevant protein fraction.

Examples provided below demonstrate (e.g. paclitaxel and cyclosporine A) that they show a higher binding to a plasma protein fraction in the absence of other excipients (such as stabilisers, ionic components, salts etc.). However there are other active substances (e.g. amphotericin B and propofol) which did not show any interference with the binding of e.g. the protein stabilisers.

Thus the proper aggregation state of the protein used has to be established for each and every pair of active substance / protein which is used according to the present invention.

When using the pair paclitaxel and HSA: it is important to eliminate all stabilisers accompanying commercially available HSA: such as N-acetyl-D,L-tryptophane, alkali caprilates which were used to stabilize the protein during pasteurisation at 60 °C. Amphotericin B or propofol can be bound to HSA also in the presence of these stabilisers. In certain instances, when the desired aggregation state could be reached by water, the other components had to be removed, following e.g. the procedure detailed below in one of the Examples.

The following aspects have to be considered for optimum combination of specific substances with specific plasma protein fractions according to the invention:

a) the characteristics of the binding site occupied by the substance on the protein;

b) possible other components present in the solution occupying the same binding site or even competing for it;

c) the physico-chemical conditions for the conformation of the actual binding site and the consequence to the binding;

d) known therapeutic aspects e.g.

i) paclitaxel on HSA having unic transport characteristics;

ii) paclitaxel on interleukines with proven therapeutic activity of the carrier;

iii) cyclosporin A on gamma immunoglobulin with proven therapeutic activity of the carrier;

iv) ritonavir on gamma immunoglobulin with proven therapeutic activity of the carrier;

stability of the formulation.

One of the simplest aggregation controlling agent is water. Using the proper amount of water unwanted aggregation may be inhibited and the protein is ready to be used according to the invention - it is in "controlled aggregation form".

According to an embodiment of the invention the compounds and compositions may contain as additive a protein aggregation controller or stabilizer and/or solution stabilizing auxiliary additive. Examples for such additives are the following: water, sodium chloride, a buffer, a poly-alcohol such as glycerol, a water-soluble sugar derivative preferably mannitol, sorbitol and/or dulcitol and others.

A further object of the present invention includes the process for the preparation of the new products and the pharmaceutical formulations according to the invention. The process comprises the following steps:

a) dissolving the therapeutically active compound having low aqueous solubility and a substantial binding affinity to plasma proteins ("active substance") in a water-miscible, pharmaceutically acceptable organic solvent,

5 b) combining said solution with the aqueous solution of a plasma protein fraction in controlled aggregation state and optionally

c) a further pharmaceutically acceptable auxiliary additive - such as a protein aggregation controller and/or a
10 stabilizer -

whereby a true solution is obtained containing the said active substance and the said protein fraction bound together by way of non-covalent bonds;

d) removing the organic solvent preferably by
15 ultrafiltering, dialysing, diafiltrating and/or lyophilising the solution or its concentrate or by combination of these treatments

whereby a homogeneous, water-soluble liquid or solid product or pharmaceutical formulation is obtained containing
20 the active substance and the plasma protein fraction;

e) optionally dissolving or diluting the solid or liquid with water whereby a clear, liquid composition is obtained which is suitable for therapeutical administration
and

25 f) optionally finishing this product into a parenteral formulation (dosage form) for direct use.

When preparing the new homogenous solid products consisting of the active substances and the proteins bound by way of non-covalent bonds according to the invention it is
30 preferable to use the process comprising the following steps according to the invention:

a) dissolving the therapeutically active compound in a water-miscible, pharmaceutically acceptable organic solvent,

b) combining said solution with the aqueous solution of the selected plasma protein fraction in controlled

5 aggregation state

whereby a true solution is obtained containing the said active substance and the said protein fraction bound together by way of non-covalent bonds;

c) removing the organic solvent and lyophilising the
10 solution or its concentrate.

The proper way to best eliminate the organic solvent depends on the active substance and on the protein involved. It follows from the nature of the active product (the pair including the active substance and the protein) that the

15 methods applied have to ensure mild conditions.

Lyophilisation leads to homogeneous, solid state water-soluble products which on redissolution in water can be administered intraperitoneally. It might be advantageous to combine the above steps e.g. to make the process more

20 economical by first preparing a concentrate of the active substance/protein pair and thereafter subjecting said concentrate to lyophilisation. Some of the active

substance/protein pairs (e.g. the pair amphotericin B/serum albumin) can be successfully concentrated by way of

25 ultrafiltration or dialysis. Some other pairs (e.g. paclitaxel/HSA) are preferably treated by way of lyophilisation. Some pairs should first be ultrafiltrated and the concentrate obtained should then be subjected to lyophilisation.

30 It is clear for the expert in the field that on the course of preparation of parenteral pharmaceuticals dilution with water includes dilution with such aqueous solutions which contain further parenterally acceptable additives such as e.g. sodium chloride.

35 The proper solvent to be used according to the invention to dissolve the active ingredient according to step a) above should have the following properties:

- it should be capable to completely dissolve the active ingredient in its mixture with water and

40 • its mixture with >50% of water should not denaturalize the protein employed.

Before starting to carry out the process according to the invention using the active ingredient and the protein selected the adequate solvent has to be determined on the basis of the above. It is suitable to use solvents where
5 mixtures containing >50% of water are still capable to dissolve the active ingredient.

Preferred solvents which can be used for step a) of the above process are for example any of the group consisting of an aliphatic C₍₂₋₄₎ monoalcohol or polyalcohol, 70 - 100%
10 ethanol, dimethyl formamide, methyl formamide.

When preparing the solution containing the protein an aggregation controller and/or solution stabilizer might be present. Such additives include a further or optimal amount of water. They also include agents capable to partially
15 occupy some of the binding sites of the protein to avoid aggregation such as any of the following agents: sodium chloride, a buffer, a poly- alcohol such as glycerol and/or a water-soluble sugar derivative preferably mannitol, sorbitol, dulcitol.

When selecting the optimal conditions in the case of any active ingredient the optimal binding affinities and corresponding aggregation properties have to be determined by preliminary measurements. In the examples below we disclose the full method of such determinations.

According to a preferred embodiment of the invention the compounds used in step a) are paclitaxel and a component of the natural plasma such as serum albumin, an immunoglobulin, glycoprotein, interferon and/or interleukin or recombinants of the same are used. Further embodiments
25 according to the invention include to use as the active substance a water-insoluble cytostatic such as a taxanoid, antibiotic, vitamin, antiinflammatory, analgesic, anticonvulsant, immunosuppressant, antiepileptic, anxiolytic, hypnotic, antifungal agent, anticoagulant, lipid peroxidase
30 inhibitor, coronary vasodilator, antiarrhythmic agent, cardiotonic, uricosuric, antithrombotic, steroid hormone (progestogen, androgen, testogen) and/or photosensitizer. Preferred active substances that can be used for the process according to the invention include the following:
40 amphotericin B, an adriamycin analogue, apazone, azathioprine, bromazepam, camptothecin, carbamazepine,

clonazepam, cyclosporine A, diazepam, dicumarol, digitoxine, dipyridamole, disopyramide, flunitrazepam, gemfibrozil, ketochlorin, ketoconazole, miconazole, niflumic acid, oxazepam, phenobarbital, phenytoin, progesterone, propofol, 5 ritonavir, sulfinpyrazone, suprofen, tacrolimus, tamoxifen, taxonoid, testosterone, tirilazad, trioxsalen, valproic acid and/or warfarin.

A preferred embodiment of the invention consists in the preparation of a homogeneous, solid, water-soluble 10 product consisting of paclitaxel and human serum albumin where the active ingredient and the plasma protein fraction can be in a non-covalent binding. A further preferred embodiment of the invention consists in the preparation of a homogeneous, solid, water-soluble product consisting of a 15 taxonoid of the general formula I and a plasma protein fraction where the active ingredient and the plasma protein fraction are in a non-covalent binding.

It is clear from the above explanations that the present invention is not limited to any of the active 20 substances nor to any of the proteins enlisted above.

A further object of the invention comprises the method of use of the products and formulations according to the invention for treatment of human or veterinary patients. The method consists in administering to a patient in need of a 25 treatment with the active ingredient an effective dose of the composition according to or prepared according to the invention. The doses that have to be applied depend on the active ingredient as well as on the protein used. Doses can be administered to ensure at least the same blood levels 30 which are known to be effective when the specific known active substances are used via other administration routes.

There is provided a preferred method of parenteral treatment of human or veterinary patients with a water-insoluble therapeutically active substance having substantial 35 affinity for binding to plasma protein by way of parenterally administering to a patient in need of a treatment with said active substance an effective dose of the following products preferably using the following dose ranges respectively (calculated on the active substance): 40 paclitaxel/albumin 70 - 280 mg/treatment; propofol/albumin 6 - 10 mg/kg/hour; camptothecin/albumin, gemfibrozil/albumin,

cyclosporin A/ albumin 3 - 5 mg/kg/day; amphotericin B/ albumin up to 1.5 mg/kg/day, whereby the same dose ranges are used for compounds containing the recombinant proteins respectively.

- 5 The compounds, compositions and methods of the invention present advantages including the following:

- it becomes possible to avoid the use of biologically incompatible vehicles, to diminish or totally avoid dose limiting side effects, related to such components like
10 toxic solvents, surface-active agents, emulsifiers and the like
- the use of plasma protein fractions as drug vehicles presents no additional toxic effects - to the contrary they may improve the tolerance of the patients e.g.
15 in the case of chemotherapy
- in desired cases the applied dose can be increased as compared with the drugs now marketed presenting thus a possibility to improve the overall outcome of therapy.

The present invention is illustrated in a more detailed manner in the following examples without the intention of limitation:

EXAMPLES

5

I. PREPARATIVE METHODS, ASSAYS

The following methods were applied to determine the binding of a particular active ingredient (substance) to a protein:

10 a) Ultrafiltration

A 1 ml sample of the clear solution formed by admixture of the aqueous solution containing the protein in controlled aggregation state and the solution of the active ingredient in an appropriate solvent is filtered through an ultrafiltration
15 membrane (cut off limit >30000 Da) and the active ingredient is determined in the ultrafiltrate fraction. When measuring the active ingredient concentration in the unfiltered solution the total amount (>90%) is recovered in unchanged form.

b) Lyophilisation

20 1 ml of the above solution is lyophilised. After lyophilization the solid residue is dissolved in about 1.00 ml of distilled water, giving a clear solution. Measuring the active ingredient concentration of this solution no active ingredient is found in the water phase but 100% is recoverable
25 from the protein fraction.

c) Analysis of the Active Ingredient

The assays for the determination of the active ingredient are done by HPLC with detection by UV spectroscopy.

The HPLC analysis can be carried out e.g. on a Waters
30 Millennium (Waters, MA, USA) HPLC system. Its components are: Waters 616 pump; Waters 600S controller; Waters 717 plus automatic sample injector, with thermostat set to +5°C; Waters 996 diode array UV/VIS detector. The system is driven and the data acquisition done by Waters Millennium v.2.02.0 run on a
35 Digital P486/166 (Digital Equipments, Irvin, UK) personal computer. The conditions have to be optimised individually for each compound, as exemplified below for several products.

d) Proof of the Chemical Structure

The LC/MS method is used to prove that the chemical
40 structure of the substance recovered from the bound fraction remained unchanged. The LC/MS assays are performed on a Finnigan Navigator (Finnigan, Manchester, UK) single

quadrupole LC/MS mass spectrometer using the ES or APCI + ionisation mode, with a MassLab v.2.0 data acquisition system run on a Digital Venturis FX/166 (Digital Equipments, Irvin, UK) personal computer. The applied conditions have to be optimised individually for each specific substance, based on the references - as exemplified in several of the following examples.

e.) Preparation of Samples

- The following is a typical sample preparation method, used to determine the total concentration/amount of a substance from a sample by HPLC and/or LC/MS analysis.

The solid content of lyophilisation vial is reconstituted with water, the solution is mixed with absolute ethanol in a ratio of 1:1 by volume, precipitating the plasma proteins, while the substances dissolved. After a quick centrifugation, the solution is suitable for HPLC or LC/MS analysis. In LC/MS it is analysed by direct sample introduction or through HPLC by way of separating the components from one another. Both methods give valuable information about the chemical structure of the parent compound and/or the possible degradation products, as exemplified in more detailed manner for several products below.

The chromatographic and mass spectroscopic data from the HPLC and LC/MS studies can confirm the chemical equivalence between the known biologically active substance used as the starting material and the compound recovered after having been bound to a protein fraction according to the invention.

f.) Materials Used

- All active substances used were of USP XXIII quality. The following plasma protein fractions were used in the experiments: (* = Ph. Eur. quality)

Human Albumin 20% sol.*	HUMAN Rt., Gödöllő, Hungary
Recombunin TM 25%	DELTA Biot.Ltd, Nottingham, UK
Humanalbumin 20% *	Biotest Ph., Dreieich, Germany
Albumeon USP	Centeon Bio-Services, Little Rock, AR, USA
Human Albumin 20% Behring*	Centeon Ph. GmbH, Wien, Austria
Human Gamma Globulin 16%*	HUMAN Rt. Gödöllő, Hungary

II. PREPARATION and CHEMICAL or PHYSICAL ASSAYS

In the following examples the plasma protein : substrate binding ratios are in the average range falling between 1 : 0.1 - 100. The substance : HSA binding ratios were calculated based on the assumption for HSA $mw = 66500$, and human gamma globulin $mw = 150000$ [see 11 Science, VOL. 244. P.1195-1198, 1989; Vox Sang, 70: p.203-209, 1996]

Example II.1

- 10 The 4.0 % (3.08×10^{-3} M) solution of human serum albumin in controlled aggregation state and the 1 mg/ml (1.17×10^{-3} M) solution of paclitaxel in absolute ethanol were admixed in 4:1 ratio and stirred so as to obtain a clear solution.

- 15 The solution is lyophilised; the solid residue is redissolved in sufficient water to ensure a clear solution having the concentration of 20% for human serum albumin. The binding is determined from UF filtrate and retentate fractions, showing 99% binding of paclitaxel to human serum albumin. This represents a 1: 0.1 ratio of human serum
20 albumin : paclitaxel.

Example II.2

- The 4.44 % (6.67×10^{-4} M) solution of human serum albumin in controlled aggregation state and the 2.0 mg/ml (2.34×10^{-3} M) solution of paclitaxel (mw 853.92) in absolute ethanol are
25 mixed in a 9:1 ratio and stirred until a clear solution is obtained. The solution is further treated as described in Example II.1.

- The binding is determined from UF filtrate and retentate
30 fractions, showing 99% binding of paclitaxel to human serum albumin. This represents a 1: 0.39 ratio of human serum albumin : paclitaxel.

Example II.3

- 35 The 4.44 % (6.67×10^{-4} M) solution of recombinant human serum albumin in controlled aggregation state and the 2.0 mg/ml (1.40×10^{-3} M) solution of paclitaxel in absolute ethanol are mixed in 9:1 ratio and stirred obtaining a clear solution.

- The solution was lyophilised; the solid residue was
40 redissolved in sufficient water to ensure a clear solution having the concentration of 20% for recombinant human serum albumin. The binding is determined from UF filtrate and

retentate fractions, showing 99% binding of paclitaxel to recombinant human serum albumin. This represents a 1 : 0.24 ratio of recombinant human serum albumin : paclitaxel.

5 **Example II.4**

A 2.25 % (1.5×10^{-4} M) solution of human gamma globulin in controlled aggregation state and a 0.1 mg/ml (1.171×10^{-4} M) solution of paclitaxel in absolute ethanol are admixed in a 9:1 ratio and stirred until a clear solution is obtained.

10 The solution is lyophilised; the solid residue is redissolved in enough water to ensure a concentration of 16 % for human gamma globulin, obtaining a clear solution.

The binding is determined from UF filtrate and retentate fractions, showing 98% binding of paclitaxel to human gamma globulin. This represents a 1: 0.71 ratio of human gamma globulin : paclitaxel.

In the above Examples II.1 through II.3 the quantity of paclitaxel was measured by HPLC following the method:

20 column MN Nucleosil C₁₈ 5µm 250x2 mm
mobile phase acetonitrile : water = 73 : 27
flow rate 0.30 ml/min
temperature ambient
detection at 273 nm
typical retention time 5.9 min; $k' = 2.93$

25 The substance was determined and found unchanged by LC/MS [see Rapid Communications in Mass Spectrometry VOL.11: p 1025-1032, 1997. and Rapid Communications in Mass Spectrometry, VOL. 9, p.495-502, 1995.]. The comparative results are shown in Figures 6: Figure 6A shows the mass spectrum of the standard, Figure 6B shows the curve of the re-dissolved sample. Figure 6C shows the fragmentation of paclitaxel.

30 LC/MS parameters: ionisation: APCI + interface; nitrogen gas flow rate: 300 l/h; solvent: acetonitrile : buffer = 60 : 40, where the buffer is 10 mM ammonium formate pH 5.0 adjusted with 10% formic acid; flow rate: 0.300 ml/min.

Assay for the Determination of Paclitaxel:

40 A C-18 reverse phase HPLC method was applied for the quantitative determination of paclitaxel from different solutions of Examples II. 1 through II. 27. The samples were injected into the HPLC system in ≥ 50 % ethanol solution, preventing any precipitation of the substance.

Binding

The binding of the substance to plasma proteins is determined after 15 minutes equilibration at $8 \pm 2^\circ\text{C}$.

- 5 The distribution of the substance is measurable after ultrafiltration through an appropriate membrane (cut-off must be $>$ than the M_w of the protein), determining the substance concentration in the ultrafiltrate fraction (representing the unbound) and in the prefiltered solution, releasing the bound part upon denaturation of the protein (representing the total). To denature the protein and release the bound fraction pre-cooled ($8 \pm 2^\circ\text{C}$) absolute ethanol is used in 1:1 ratio. The exact concentration values and amounts are calculated in consideration of the dilution factor.

15 Examples II.5 to II.21

- The solution of human serum albumin in the concentration range of 20% ($3.08 \times 10^{-3} \text{ M}$) to 0.02% ($3.08 \times 10^{-6} \text{ M}$) is combined with the solution of paclitaxel in absolute ethanol in the concentration range from 20 mg/ml ($2.34 \times 10^{-2} \text{ M}$) to 0.01 mg/ml ($1.17 \times 10^{-5} \text{ M}$) obtaining always clear solutions. Details are presented in Table I. All measurements are performed three times and the calculated results are averaged.

TABLE I

Example	$[T]_T$ (mM)	[HSA] (mM)	$n(T_0)/n$ (HSA)	$n(T_0)/n/T_T \times 100\%$
II.5	0.2342	2.410	0.093	97.4
II.6	0.2342	1.205	0.177	93.2
II.7	0.2342	0.602	0.346	91.0
II.8	0.2342	0.301	0.648	85.2
II.9	0.2342	0.121	1.545	81.2
II.10	0.2342	0.0602	3.125	82.1
II.11	0.2342	0.0241	5.662	59.5
II.12	0.2342	0.0121	4.948	26.0
II.13	0.2342	0.00602	5.823	15.3
II.14	0.2342	0.00241	10.419	11.0
II.15	0.2342	0.00121	14.367	7.6
II.16	0.2342	0.000602	12.370	3.3
II.17	4.6843	0.121	4.135	10.9
II.18	2.3421	0.121	8.401	44.2
II.19	1.1711	0.121	4.585	48.2
II.20	0.4648	0.121	2.864	75.3
II.21	0.1171	0.121	0.765	80.4

Legend:

- [T]_T total paclitaxel concentration after addition to human serum albumin
- [HSA] concentration of human serum albumin
- 5 $n(T_b)/n(HSA)$ number of moles of paclitaxel bound per mole of human serum albumin
- $n(T_b)/n(T_T) \times 100\%$ percentage of bound paclitaxel.

- Variation of paclitaxel concentration (with 0.08 % HSA, 10 % ethanol, 0.002 mg/ml paclitaxel) is shown on Figure 7; variation of albumin concentration (with 0.004 - 16.0 % HSA, 20 % ethanol, 0.2 mg/ml paclitaxel) is shown on Figure 8. variation of paclitaxel binding to HSA (with 0.8 % HSA, 10 % ethanol, 0.1 to 2.0 mg/ml paclitaxel) as a function of pH at values of pH 4.0 to 8.5 is shown on Figure 9. The signs on the graph correspond to the following examples:

- Example II.18 -♦-♦-
- Example II.19 -O-O-
- 20 Example II.20 -x-x-
- Example II.15 -◇-◇-
- Example II.21 -▲-▲-

Example II.22

- 25 Similar methods as above in Examples II.2 to II.21 are used with animal serum albumin, immunoglobulin, glycoproteides, interferons and interleukines.

Example II.23

- 30 Treatment of commercially available human serum albumin or recombinant human serum albumin (in the following albumin) to achieve the controlled aggregation state with the best binding conditions of the molecule include removal of stabilisers, such as sodium caprylate, N-acetyl-D,L-tryptophan and other ionic components and salts.

a.) Ultrafiltration Method

- Adjust the pH of the solution containing 10% albumin to 3.0 with hydrochloric acid and dilute to 5% protein content with bi-distilled water. Concentrate the solution to 10% for protein content using ultrafiltration (membrane cut off limit 30000 kD).
- 40

Dilute the solution back to 5% protein content with 1.0 mM hydrochloric acid. Concentrate the solution to 10% protein content using ultrafiltration (membrane cut off limit 30000 kD).

- 5 Repeat the procedure 12x, then adjust the pH to 6.9 with a 2.0 M aqueous sodium hydroxide solution and dilute the solution to 5% concentration for protein content with bi-distilled water. Concentrate the solution to 10% for protein content using ultrafiltration (membrane cut off limit 30000 kD) again.

- 10 Dilute the solution back to 5% for protein content with bi-distilled water. Concentrate the solution to 10% for protein content using ultrafiltration (membrane cut off limit 30000 kD). Repeat the procedure 10x, obtaining a pure protein fraction, sufficiently free from other excipients. By that time, the conductivity of the ultrafiltrate is close to that of the bi-distilled water used for dilution. This protein is adequate for use to bind e.g. paclitaxel or cyclosporin.

b.)

- 20 Instead of ultrafiltration the use of dialysis gives similar results. The treatment requires about 48 hours.

Example II.24

- 25 The 0.8 % ($1.203 \cdot 10^{-4}$ M) solution of HSA and the 4.0 mg/ml ($4.33 \cdot 10^{-3}$ M) solution of amphotericin B (mw= 924.09) in DMF were mixed in a 9:1 ratio and stirred obtaining a clear solution.

- The solution was lyophilised; the solid residue was redissolved using enough water to ensure that the concentration was 20% for HSA, obtaining a clear solution. The binding was determined from UF filtrate and retentate fractions, showing 99.7% binding of amphotericin B to HSA. This represents a 1:4 ratio for HSA : amphotericin B.

- 35 **Example II.25**

The 0.8 % ($1.203 \cdot 10^{-4}$ M) solution of recombinant human serum albumin and the 40.0 mg/ml ($4.33 \cdot 10^{-2}$ M) solution of amphotericin B in DMF + HCl are mixed in a 9:1 ratio and stirred obtaining a clear solution.

- 40 The solution is lyophilised; the solid residue is redissolved in sufficient water to make the final concentration 20% for recombinant HSA, obtaining a clear

solution. The binding is determined from the UF filtrate and retentate fractions, showing 99.5% binding of amphotericin B to HSA. This represents a 1:40 ratio for recombinant HSA : amphotericin B.

- 5 Amphotericin B is measured by HPLC following the method below:

column MN Nucleosil C₁₈ 5µm 250x2 mm
mobile phase acetonitrile : buffer =1:1
(buffer : 0.2% formic acid pH adjusted to
10 4.0 with triethylamine)
flow rate 0.30 ml/min
temperature ambient
detection at 365 nm
typical retention time 5.3 min, $k' = 1.41$

- 15 The substance is determined and found unchanged using LC/MS. The comparative results are shown in Figures 2: Figure 2A shows the mass spectrum of the standard, Figure 2B shows the curve of the re-dissolved sample. Figure 2C shows the fragmentation of amphotericin B.

- 20 LC/MS parameters: ionisation: ESI + interface;
nitrogen gas flow rate: 300 l/h; solvent: 20 mM ammonium formate pH 4.0 adjusted with 10% formic acid; flow rate: 0.300 ml/min.

25 Example II.26

- The 0.4% (6.015×10^{-5} M) solution of HSA in controlled aggregation state and the 0.14 mg/ml (4.02×10^{-4} M) solution of camptothecin (mw= 348.36) in abs. ethanol were admixed in a 4:1 ratio and stirred to obtain a clear solution. The solution was lyophilised; the solid residue was redissolved in
30 enough water to ensure that the final concentration was 20% for HSA, thereby obtaining a clear solution. The binding was determined from the UF filtrate and retentate fractions, showing 98% binding of camptothecin to HSA. This represents a
35 1:5.34 ratio of HSA : camptothecin.

Example II.27

- The 0.4% (6.015×10^{-5} M) solution of recombinant HSA in controlled aggregation state and the 0.14 mg/ml (4.02×10^{-4} M) solution of camptothecin in abs. ethanol were mixed in 4:1
40 ratio and stirred obtaining a clear solution.

The solution was lyophilised; the solid residue was redissolved in that much water as the final concentration was 20% for recombinant HSA, obtaining a clear solution. The binding was determined from UF filtrate and retentate fractions, showing 98% binding of camptothecin to HSA. This represents a 1:5.34 ratio of recombinant HSA: camptothecin.

We measured the camptothecin by HPLC as follows:

column MN Nucleosil C₁₈ 5µm 250x2 mm

mobile phase acetonitrile : buffer=33:67

flow rate 0.33 ml/min

temperature ambient

detection at 356 nm.

typical retention time 6.9 min $k' = 2.45$

The substance was determined and found unchanged by

LC/MS [Cancer Research, VOL.56: p.3689-3694, 1996.]

Example II.29

The 4.0 % (6.015*10⁻⁴ M) solution of HSA in controlled aggregation state and the 8.0 mg/ml (3.39*10⁻² M) solution of carbamazepin (mw 236.27) in abs. ethanol were admixed in 19:1 ratio and stirred obtaining a clear solution. The solution was lyophilised; the solid residue was redissolved in a sufficient amount of water to make the final concentration 20% for HSA, obtaining a clear solution. The binding was determined from UF filtrate and retentate fractions, showing 98 % binding of carbamazepin to HSA. This represents a 1:2.8 ratio of HSA : carbamazepine.

The carbamazepin was measured by HPLC following the method below:

column MN Nucleosil C₁₈ 5µm 250x2 mm

mobile phase acetonitrile : buffer=1:1

(buffer: 0.2% formic acid pH adjusted to 7.0 with triethylamine)

flow rate 0.25 ml/min

temperature ambient

detection at 285 nm

typical retention time 5.3 min $k' = 1.12$

The substance was determined and found unchanged by

LC/MS [Eur. J. Clin. Chem Clin. Biochem. 35(10): p:755-759, 1997]. The comparative results are shown in Figures 3: Figure 3A shows the mass spectrum of the standard, Figure 3B

shows the curve of the re-dissolved sample. Figure 3C shows the fragmentation of carbamazepin.

LC/MS parameters: ionisation: ESI + interface; nitrogen gas flow rate: 300 l/h; solvent: 2 mM ammonium formate; flow rate: 0.250 ml/min.

Example II.30

The 4.0% (6.015×10^{-4} M) solution of HSA in controlled aggregation state and the 1.0 mg/ml (8.33×10^{-4} M) solution of cyclosporine A (mw 1202.63) in absolute ethanol were mixed in 9:1 ratio and stirred obtaining a clear solution.

The solution was lyophilised; the solid residue was redissolved in a sufficient amount of water to make the final concentration 20% for HSA, obtaining a clear solution. The binding was determined from UF filtrate and retentate fractions, showing 97 % binding of cyclosporine A to HSA.. This represents a 1:0.14 ratio for HSA : cyclosporine A.

Example II.31

The 2.0% (3.008×10^{-4} M) solution of recombinant HSA in controlled aggregation state and the 1.0 mg/ml (8.33×10^{-4} M) solution of cyclosporine A in absolute ethanol were mixed in 9:1 ratio and stirred obtaining a clear solution.

The solution was lyophilised; the solid residue was redissolved in a sufficient amount of water to make the final concentration 20% for recombinant HSA, obtaining a clear solution. The binding was determined from UF filtrate and retentate fractions, showing 98% binding of cyclosporine A to recombinant HSA. This represents a 1 : 0.29 ratio for recombinant HSA : cyclosporine A.

Example II.32

The 2.25% (1.50×10^{-4} M) solution of human gamma globulin and the 1.0 mg/ml (8.33×10^{-4} M) solution of cyclosporine A in absolute ethanol were mixed in a 9:1 ratio and stirred obtaining a clear solution.

The solution is lyophilised; the solid residue is redissolved in enough water to give a concentration of 16% for human gamma globulin, thereby obtaining a clear solution. The binding is determined from the UF filtrate and retentate fractions, showing 98 % binding of cyclosporine A to human

gamma globulin.. This represents a 1 : 0.56 ratio for human gamma globulin : cyclosporine A.

The cyclosporine A was measured by HPLC following the method below:

5 column MN Nucleosil C18 5µm 250x2 mm
 mobile phase acetonitrile:water : methanol :
phosphoric acid = 700:260:40:0.05
 flow rate 0.350 ml/min
 temperature 80 °C thermostat
10 detection at 205 nm
 typical retention time 7.5 min k' = 2.95

The substance was determined and found unchanged by LC/MS [1], as the results show. The comparative results are shown in Figures 4: Figure 4A shows the mass spectrum of the standard, Figure 4B shows the curve of the re-dissolved sample. Figure 4C shows the fragmentation of cyclosporine A.

LC/MS parameters: ionisation: ESI + interface, nitrogen gas flow rate: 300 l/h; solvent: acetonitrile/ water = 60 / 40; solvent flow rate: 0.350 ml/min.

20

Example II.33

The $0.4 \mu\text{g}$ ($6.015 \cdot 10^{-5}$ M) solution of HSA and the 2.0 mg/ml ($1.12 \cdot 10^{-2}$ M) solution of propofol (mw178.27) in absolute ethanol were mixed in 9:1 ratio and stirred obtaining a clear solution.

25

The solution was lyophilised; the solid residue was redissolved in a sufficient amount of water to make the final concentration 20% for HSA, obtaining a clear solution. The binding was determined from UF filtrate and retentate fractions, showing 99% binding of propofol to HSA. This represents a 1:18.3 ratio of HSA : propofol.

30

Example II.34

The $0.4 \mu\text{g}$ ($6.015 \cdot 10^{-5}$ M) solution of recombinant HSA and the 2.0 mg/ml ($1.12 \cdot 10^{-2}$ M) solution of propofol in absolute ethanol were mixed in 9:1 ratio and stirred obtaining a clear solution.

35

The solution is lyophilised; the solid residue was redissolved in a sufficient amount of water to make the final concentration 20% for recombinant HSA, obtaining a clear solution. The binding was determined from UF filtrate and retentate fractions, showing 99% binding of propofol to

40

recombinant HSA. This represents a 1 : 18.3 ratio of recombinant HSA : propofol.

Propofol was measured by HPLC as follows:

5 column MN Nucleosil C18 5µm 250x2 mm
 mobile phase acetonitrile : water=73:27
 flow rate 0.30 ml/min
 temperature ambient
 detection at 273 nm
 typical retention time 6.1 min $k' = 1.77$

10 The substance was determined and found unchanged by LC/MS [J.of Chromatography B, 669: p. 358 - 365, 1995]. The comparative results are shown in Figures 5: Figure 5A shows the mass spectrum of the standard, Figure 5B shows the curve of the re-dissolved sample. Figure 5C shows the fragmentation
15 of propofol.

LC/MS parameters:

ionisation: APCI + interface; nitrogen flow rate: 300 l/h;
solvent: acetonitrile / water = 73 / 23; flow rate: 0.300
ml/min.

20

Example II.35

9.0 ml of a 0.8% ($1.213 \cdot 10^{-4}$ M) solution of HSA and 1.0 ml of a 4.0 mg/ml ($4.33 \cdot 10^{-3}$ M) solution of amphotericin B in dimethyl formamide were mixed to give a clear solution. This
25 solution was dialyzed against 2.0 liter of water (WFI) at 4 °C for period of 20 hours protected from light.

Using the determination method of Example II.24 the binding was found to be 99.6% representing a 1 : 3.5 ratio for HSA: amphotericin B.

30 On repeating the dialysis procedure five times the concentration of DMF in the solution was reduced below its detection limit ($2 \cdot 10^{-9}$ M).

III. DOSAGE FORMS

Examples III.1 to III.6

Following the procedure for the preparation with lyophilisation as described above an appropriate pharmaceutical formulation is obtained. Re-dissolving the solid in adequate volume of WFI so as to reach the concentration of 20% for HSA the solution arrives to a concentration suitable for therapeutic application as summarised below for some active substances:

Example	name.	conc. mg/ml
III.1	amphotericin B	11.09
III.2	camptothecin	6.8
III.3	carbamazepine	1.98
III.4	cyclosporin A	0.50
III.5	paclitaxel	1.0
III.6	propofol	10.0

The above dosage forms can be further finished in vials for injectables and infusions.

IV. BIOLOGICAL EXAMPLES

Studies on Biological Equivalence

Biological equivalence was determined comparing the new formulations according to the invention with known formulations used in therapy containing the same active substance with poor water solubility. Such known formulations were prepared in polyoxyethylated castor oil (Cremophor EL) and absolute ethanol.

Materials used:

Paclitaxel dissolved in a mixture of polyoxyethylated castor oil (Cremophor EL): absolute ethanol = 1:1, was compared with the aqueous solution of paclitaxel/HSA of the invention, prepared according to Example II.2.

Example IV.1 In vitro studies

Comparative studies were carried out in vitro to determine the antiproliferative and cytotoxic activity on human tumour cell lines. The Cremophor EL/absolute ethanol and HSA formulation of paclitaxel was compared on K562 human myeloid leukaemia, MCF-7 and MDA-231 breast and OVCAR-5

ovarian carcinoma cell lines [Anticancer Research, Vol. 16:
p.2469-2478, 1996.]

Method:

Colony growth inhibition assay: Monolayer cultures of the cell
lines were treated with eight different concentrations of the
drug in the two above formulations plus in DMSO/saline
solution as a reference. The cultures were incubated for 24,
48, 72, 96 and 120 hours respectively. The colonies were
stained with crystal violet and the survival of treated cells
was calculated as percentage of colonies formed by untreated cells.
Tables II A through IV B show the results obtained on
the different cell lines. In each study the survival of
treated cells is shown, calculated as percentage of colonies
formed by untreated cells. All values are the average of three
experiments.

TABLE II A

cell line: MCF7 breast carcinoma

formulation: paclitaxel/Cremophor EL & absolute ethanol

Ptx cc[μ M] \ t[h]	24h	48h	72h	96h	120h
0.005	92*	86	76	42	30
0.01	90	81	72	33	26
0.02	86	71	67	29	23
0.025	84	64	60	24	18
0.05	82	60	52	23	16
0.1	80	57	38	18	15
1.0	68	46	28	15	6.5
10.0	62	33	21	10	4.6

TABLE II B

cell line: MCF7 breast carcinoma

formulation: paclitaxel/HSA

Ptx cc[μ M] \ time [h]	24h	48h	72h	96h	120h
0.005	91	84	75	41	27
0.01	88	81	69	35	23
0.02	84	76	64	31	20
0.025	80	70	59	28	16
0.05	77	66	53	25	12
0.1	75	59	46	20	9.5
1.0	67	42	30	17	6.2
10.0	58	31	21	9.0	3.0

TABLE III A

cell line: MDA-231 breast carcinoma

formulation: paclitaxel/Cremophor EL & absolute ethanol

Ptx cc[μ M] \ time [h]	24h	48h	72h	96h	120h
0.005	97	89	80	47	34
0.01	94	87	75	41	30
0.02	89	82	69	37	28
0.025	86	76	65	34	23
0.05	84	72	59	29	21
0.1	83	66	53	26	18
1.0	73	49	34	21	9.5
10.0	65	37	24	14	8.2

5

TABLE III B

cell line: MDA-231 breast carcinoma

formulation: paclitaxel/HSA

Ptx cc[μ M] \ time [h]	24h	48h	72h	96h	120h
0.005	92	78	51	30	10
0.01	86	65	38	24	8.3
0.02	75	51	33	22	7.0
0.025	64	47	28	19	6.4
0.05	60	42	26	18	5.3
0.1	55	36	24	16	4.0
1.0	49	33	22	15	3.2
10.0	45	26	20	10	2.6

10

TABLE IV A

cell line: K562 human myeloid leukaemia

formulation: paclitaxel/Cremophor EL & absolute ethanol

Ptx cc[μ M] \ time [h]	24h	48h	72h	96h	120h
0.005	88	59	30	21	10
0.01	79	40	21	15	8.7
0.02	66	31	19	12	7.2
0.025	62	29	17	10	6.0
0.05	56	25	14	9.4	5.4
0.1	51	23	12	7.7	4.6
1.0	47	20	10.5	6.0	3.0
10.0	39	16	9.5	4.2	2.0

TABLE IV B

cell line: K562 human myeloid leukaemia sample: paclitaxel/HSA

Ptx cc[μM] \ time [h]	24h	48h	72h	96h	120h
0.005	89	53	31	18	5.4
0.01	75	40	22	11	4.7
0.02	69	32	18	9.0	4.0
0.025	65	30	14	7.6	3.5
0.05	58	25	11	7.0	3.0
0.1	53	21	9.5	5.6	2.4
1.0	47	18	8.0	5.0	1.7
10.0	41	16	7.1	4.7	1.0

Example IV.2: In Vivo Pharmacokinetic Test

From the therapeutic point of view, the bio-equivalence

- 5 can be considered, demonstrating equal pharmacokinetic
characteristics such as AUC (area under the curve), elimi-
nation constants, plasma half life after the administration of
the same dose to the same species. Such experiment was done on
rats for the two formulations as in Example IV.1. [Semin
10 Oncol, VOL. 21 (5 Suppl. 8): p.53-62, 1994].

AUC means the area under the curve on a plasma concentration versus time diagram. It can be generated measuring the plasma concentration of the compound administered at different points of time.

15 Pharmacokinetic study on rats:

Method: The dose of 2.5 mg/kg paclitaxel was administered in 1.0 ml volume i.v.. bolus to CR. (Wi) BR rats (body weight between 380 and 420 grams), and a 1.0 ml blood sample was drawn into a heparinised test tube from three animals at each point of time as indicated below:

[illegible]

The plasma fraction was separated by quick centrifugation at +5°C and kept frozen at -70°C until processed for analytical measurement.

5 Sample preparation: The frozen plasma samples were warmed up to +8 °C, centrifuged for 5 min at 5000 RPM. 0.300 - 0.500 ml of the clear plasma solution was taken out and loaded onto an Oasis HLB 1 cc SPE (Solid Phase) Extraction cartridge. Before the plasma sample was loaded the cartridge was rinsed
10 with 1 ml of methanol, followed by 1 ml water for pre-conditioning. The paclitaxel content absorbed onto the SPE cartridge, while the rest of the sample components were rinsed out with 1 ml of water and 1 ml of 30% acetonitrile/water solution. The cartridge was blown dry by air. Paclitaxel was
15 eluted from the SPE cartridge with 1 ml absolute ethanol. The sample was evaporated to dryness with nitrogen, stored at (-20°C) for analysis. The residue was dissolved in 0.200 ml absolute ethanol and injected for HPLC analysis.

20 The HPLC conditions were the same as applied for substance identification.

Results:

The points obtained were the average of three measured from the samples of three individual animals. As a result, the difference between the two curves obtained from the
25 pharmacokinetic study for the equal dose of the two different formulations, remained within the deviation of the individual samples. The same curve takes shape plotting all the individual data, indicating no or minor difference in pharmacokinetic characteristics of the two formulations.

30

Example IV.3

In vivo evaluation of antiproliferative and cytotoxic activity investigations show that the new formulations shows a positive effect against human tumour xenografts CH1 and CH1_{tax}
35 in nude mice.

Example IV.4

Hypersensitivity Tests

About 45% of the patients treated with paclitaxel
40 expressed hypersensitivity reactions. These side effects were proven related to one excipient of the formulation, Cremophor EL, as observed with other pharmaceutical products containing

the same component. This hypersensitivity reaction is determined as anaphylactic toxicity expressed through induction of histamine release by Cremophor EL.

Our study was performed on CRL (WI) BR male rats weighing 130 - 150 g [14]. The administered dose was calculated around 7.0 mg/kg for paclitaxel, given i.v. in 1.0 ml of total volume. A group for each time point and dose contained 5 animals. The blood samples were collected into heparin containing tubes after 2, 5 and 10 minutes of treatment. The plasma was separated by quick centrifugation. The samples were stored at -70°C .

The histamine content of the sample was C¹⁴-methylated by specific histamine-N-methyl-transferase enzyme. The histamine level was determined in the plasma samples measuring the C¹⁴ radioactivity in the samples.

The data obtained indicated that Cremophor EL and the containing formulation have substantial histamine release induction, while HSA and the HSA containing formulation and paclitaxel itself do not show any such effect.

Example IV.5

The same phenomenon as in Example IV.4 was found using *in vitro* human experiments from human blood samples based on the quantitative assay of chromatin activation of blood lymphocytes [Method: Analytical and Quantitative Cytology and Histology, VOL. 8: p.1, 1986.].

CLAIMS

- 5 1. A water-soluble product or pharmaceutical
formulation in solid or liquid form mainly for parenteral
use containing
a) a therapeutically active compound having low aqueous
solubility and a substantial binding affinity to plasma
10 proteins (in the following "active substance") and
b) a plasma protein fraction in controlled aggregation
state
whereby the said active substance and the said protein fraction
are bound to each other by way of non-covalent bonds and
15 c) optionally further pharmaceutically acceptable and
mainly parenterally acceptable formulation additive(s) - such
as water, stabilizer(s), protein aggregation controller(s).
- 20 2. A water-soluble product or human pharmaceutical
formulation according to claim 1 mainly for parenteral use
containing a therapeutically active substance having low
aqueous solubility and a human plasma protein fraction in
controlled aggregation state.
- 25 3. A water-soluble product or veterinary pharmaceutical
formulation according to claim 1 mainly for parenteral use
containing a therapeutically active substance having low
aqueous solubility and an animal plasma protein fraction in
controlled aggregation state.
- 30 4. A product or pharmaceutical formulation according to
any of claims 1 to 3 containing a component of the natural -
human or animal - plasma such as serum albumin, an
immunoglobulin, glycoprotein, interferon and/or interleukin or
35 a recombinant of said plasma component.
5. A product or pharmaceutical formulation according to
any of claims 1 to 4 for human administration, mainly for
parenteral use, containing a component of the natural - human
40 plasma such as serum albumin, an immunoglobulin, glycoprotein,
interferon and/or interleukin or a recombinant of said plasma

component.

6. A product or pharmaceutical formulation according to any of claims 1 to 3 containing as the water-insoluble active substance a cytostatic such as a taxonoid, an antibiotic, vitamin, antiinflammatory, analgesic, antiviral, anticonvulsant, immunosuppressant, antiepileptic, anxiolytic, hypnotic, antifungal agent, anticoagulant, lipid peroxidase inhibitor, coronary vasodilator, antiarrhythmic agent, cardiotonic, uricosuric, antithrombotic, steroid hormone (progestogen, androgen, testosterone) and/or photosensitizer.

7. A product or pharmaceutical formulation according to any of claims 1 to 6 containing at least one of the following active substances: amphotericin B, an adriamycin analogue, apazone, azathioprine, bromazepam, camptothecin, carbamazepine, clonazepam, cyclosporine A, diazepam, dicumarol, digitoxine, dipyridamole, disopyramide, flunitrazepam, gemfibrozil, ketochlorin, ketoconazole, miconazole, niflumic acid, oxazepam, phenobarbital, phenytoin, progesterone, propofol, ritonavir, sulfipyrazone, suprofen, tacrolimus, tamoxifen, taxonoid, testosterone, tirilazad, trioxsalen, valproic acid, warfarin.

8. A product or pharmaceutical formulation according to any of claims 1 to 7 containing a taxonoid of the general formula I - in the formula
- R¹ represents tert. butyl-oxy-carboxylic acid amide or benzoyl amide
- R² represents hydrogen or any acyl group preferably acetyl.

9. A product or pharmaceutical formulation according to any of claims 1 to 7 containing paclitaxel and human serum albumin, immunoglobulin, glycoprotein, interferon and/or interleukin or some other natural or recombinant human plasma protein fraction in the molar ratio of 1 : 0.05 to 1 : 100, preferably in the molar ratio of 1 : 0.1 to 1 : 50.

10. A product or pharmaceutical formulation according to any of claims 1 to 9 containing azathioprine and human serum albumin, immunoglobulin, glycoprotein, interferon and/or

interleukin or some other natural or recombinant human plasma protein fraction in the molar ratio of 1 : 0.05 to 1 : 100, preferably 1 : 0.1 to 1 : 50.

5 11. A formulation according to any of claims 1 to 8 containing camptothecin and human serum albumin, immunoglobulin, glycoprotein, interferon and/or interleukin or some other natural or recombinant human plasma protein fraction in the molar ratio of 1 : 0.05 to 1 : 100, preferably 1 : 0.1
10 to 1 : 50.

 12. A formulation according to any of claims 1 to 8 containing gemfibrozil and human serum albumin, immunoglobulin, glycoprotein, interferon and/or interleukin or some other
15 natural or recombinant human plasma protein fraction in the molar ratio of 1 : 0.05 to 1 : 100, preferably 1 : 0.1 to 1 : 50.

 13. A product or pharmaceutical formulation according to
20 any of claims 1 to 8 containing miconazole and human serum albumin, immunoglobulin, glycoprotein, interferon and/or interleukin or some other natural or recombinant human plasma protein fraction in the molar ratio of 1 : 0.05 to 1 : 100, preferably 1 : 0.1 to 1 : 50.

25 14. A product or pharmaceutical formulation according to any of claims 1 to 8 containing propofol and human serum albumin, immunoglobulin, glycoprotein, interferon and/or interleukin or some other natural or recombinant human plasma
30 protein fraction in the molar ratio of 1 : 0.05 to 1 : 100, preferably 1 : 0.1 to 1 : 50.

 15. A product or pharmaceutical formulation according to any of claims 1 to 8 containing tamoxifen and human serum
35 albumin, immunoglobulin, glycoprotein, interferon and/or interleukin or some other natural or recombinant human plasma protein fraction in the molar ratio of 1 : 0.05 to 1 : 100, preferably 1 : 0.1 to 1 : 50.

40 16. A product or pharmaceutical formulation according to any of claims 1 to 8 containing ritonavir and human serum albumin, immunoglobulin, glycoprotein, interferon and/or

interleukin or some other natural or recombinant human plasma protein fraction the molar ratio of 1 : 0.05 to 1 : 100, preferably 1 : 0.1 to 1 : 50.

5 17. A pharmaceutical formulation according to any of claims 1 to 8 containing tacrolimus and human serum albumin, immunoglobulin, glycoprotein, interferon and/or interleukin or some other natural or recombinant human plasma protein fraction in the molar ratio of 1 : 0.05 to 1 : 100, preferably 1 : 0.1
10 to 1 : 50.

 18. A pharmaceutical formulation according to any of claims 1 to 8 containing tirilazad and human serum albumin, immunoglobulin, glycoprotein, interferon and/or interleukin or
15 some other natural or recombinant human plasma protein fraction in the molar ratio of 1 : 0.05 to 1 : 100, preferably 1 : 0.1 to 1 : 50.

 19. A pharmaceutical formulation according to any of
20 claims 1 to 8 containing trioxsalen and human serum albumin, immunoglobulin, glycoprotein, interferon and/or interleukin or some other natural or recombinant human plasma protein fraction in the molar ratio of 1 : 0.05 to 1 : 100, preferably 1 : 0.1 to 1 : 50.
25

 20. A pharmaceutical formulation according to any of claims 1 to 19 having a solid state or having the form of an aqueous solution.

 21. A pharmaceutical formulation according to any of
30 claims 1 to 20 containing as additive an agent stabilizing the solution and/or the protein.

 22. A pharmaceutical formulation according to claim 21 containing as solution and/or protein stabilizing agent any of the following: sodium chloride, a buffer, an alcohol such as
35 glycerol and/or a water-soluble sugar derivative preferably mannitol, sorbitol, dulcitol.

 23. Process for the preparation of a product or
40 pharmaceutical formulation according to any of claims 1 to 22 or a new product according to claims 30 to 37 characterized b y

- a) dissolving the therapeutically active compound having low aqueous solubility and a substantial binding affinity to plasma proteins ("active substance") in a water-miscible, pharmaceutically acceptable organic solvent,
- 5 b) combining said solution with the aqueous solution of a plasma protein fraction in controlled aggregation state and optionally
- c) a further pharmaceutically acceptable auxiliary additive - such as a protein aggregation controller and/or a
- 10 stabilizer -
whereby a true solution is obtained containing the said active substance and the said protein fraction bound together by way of non-covalent bonds;
- d) removing the organic solvent preferably by
- 15 ultrafiltering, dialysing, diafiltrating and/or lyophilising the solution or its concentrate or by combination of these treatments
whereby a homogeneous, water-soluble liquid or solid pharmaceutical product is obtained containing the active
- 20 substance and the plasma protein fraction;
- e) optionally dissolving the solid product in water or diluting the liquid product with water whereby a clear, liquid composition is obtained which is suitable for therapeutical administration and
- 25 f) optionally finishing this product into a parenteral formulation (dosage form) for direct use.

25. Process for the preparation of a product or pharmaceutical formulation according to any of claims 1 to 24
- 30 or 30 to 37 characterized b y
- a) dissolving the therapeutically active compound in a water-miscible, pharmaceutically acceptable organic solvent,
- b) combining said solution with the aqueous solution of the selected plasma protein fraction in controlled
- 35 aggregation state, said solution containing optionally
- c) a further pharmaceutically acceptable auxiliary additive - such as a protein aggregation controller and/or a stabilizer -
- whereby a true solution is obtained containing the
- 40 said active substance and the said protein fraction bound together by way of non-covalent bonds;

d) removing the organic solvent and lyophilising the solution or its concentrate.

26. A process according to step a) of any of claims 23 to 25 characterized by using to dissolve the active substance a solvent having the following properties:

- a) it is capable to completely dissolve the active substance in its mixture with water and
- b) its mixture with <50% of water does not denaturalize the protein employed.

27. A process according to claim 26 characterized by using as the solvent any of the group an aliphatic C(2-4) monoalcohol or polyalcohol, 70 - 100% ethanol, dimethyl formamide, methyl formamide.

28. A process according to step a) of claim 23 to 27 characterized by using as protein aggregation controller or stabilizer and/or solution stabilizing auxiliary additive any of the following agents: water, sodium chloride, a buffer, a poly- alcohol such as glycerol and/or a water-soluble sugar derivative preferably mannitol, sorbitol and/or dulcitol.

29. A process according to step a) of any of claims 23 to 28 characterized by using paclitaxel and a component of the natural plasma such as serum albumin, an immunoglobulin, glycoprotein, interferon and/or interleukin or a recombinant of the same.

30. A homogeneous, solid, water-soluble product consisting of at least one active substance of the group amphotericin B, an adriamicine analogue, apazone, azathioprine, bromazepam, camptothecin, carbamazepine, clonazepam, cyclosporine A, diazepam, dicumarol, digitoxine, dipyrindamole, disopyramide, flunitrazepam, gemfibrozil, ketochlorin, ketoconazole, miconazole, niflumic acid, oxazepam, phenobarbital, phenytoin, progesterone, propofol, ritonavir, sulfinpyrazone, suprofen, tacrolimus, tamoxifen, taxonoid, testosterone, tirilazad, trioxsalen, valproic acid and/or warfarin

and also consisting of at least one protein of the group human serum albumin, immunoglobulin, glycoprotein, interferon and/or interleukin or some other natural or recombinant human plasma protein fraction

- 5 where the said active substance and the said protein fraction are bound to each other by way of non-covalent bonds and wherein the molar ratio of the said active substance and the said protein fraction is within the range of 1 : 0.05 to 1 : 100, preferably of 1 : 0.1 to 1 : 50.

10

31. A homogeneous, solid, water-soluble product according to claim 30 consisting of a taxonoid of the general formula I - in the formula

- 15 R^1 represents tert. butyl-oxy-carboxylic acid amide or benzoyl amide,

R^2 represents hydrogen or any acyl group preferably acetyl - and of a plasma protein fraction.

20

32. A homogeneous, solid, water-soluble product according to claim 30 consisting of paclitaxel and human serum albumin, recombinant human plasma albumin and/or γ -globulin.

- 25 33. A homogeneous, solid, water-soluble product according to claim 30 consisting of amphotericin B and human serum albumin, recombinant human plasma albumin and/or γ -globulin.

- 30 34. A homogeneous, solid, water-soluble product according to claim 30 consisting of camptothecin and human serum albumin, recombinant human plasma albumin and/or γ -globulin.

- 35 35. A homogeneous, solid, water-soluble product according to claim 28 consisting of carbamazepin and human serum albumin, recombinant human plasma albumin and/or γ -globulin.

- 40 36. A homogeneous, solid, water-soluble product according to claim 28 consisting of cyclosporin A and human serum albumin, recombinant human plasma albumin and/or γ -

globulin.

37. A homogeneous, solid, water-soluble product according to claim 30 consisting of propofol and human serum
5 albumin, recombinant human plasma albumin and/or γ -globulin.

38. Method of treatment of human or veterinary patients with a water-insoluble therapeutically active substance having substantial plasma protein affinity characterized by
10 administering to a patient in need of a treatment with said active substance an effective dose of the product or pharmaceutical formulation according to or prepared according to any of claims 1 to 37.

15 39. Method of parenteral treatment of human or veterinary patients with a water-insoluble therapeutically active substance having substantial affinity for binding to plasma protein characterized by parenterally
20 administering to a patient in need of a treatment with said active substance an effective dose of the following products preferably using the following dose ranges respectively (calculated on the active substance): paclitaxel/albumin 70 - 280 mg/treatment; propofol/albumin 6 - 10 mg/kg/hour; camptothecin/albumin, gemfibrozil/albumin, cyclosporin A/
25 albumin 3 - 5 mg/kg/day; amphotericin B/ albumin up to 1.5 mg/kg/day, whereby the same dose ranges are used for compounds containig the recombinant proteins respectively.

40. Method for parenteral delivery in therapeutic use
30 of pharmaceutically active ingredients with poor solubility in water and substantial affinity for binding to plasma proteins characterized by administering to a patient in need of a treatment with said active substance an effective dose of the composition according to or prepared according to any of
35 claims 1 to 19.

41. A product or method substantially as described in any of the examples.

AMENDED CLAIMS

[received by the International Bureau on 05 January 1999 (05.01.99);
original claims 1,2-5,23,25,30 and 38-40 replaced by amended claims 1,3-6,24,25,30 and 38-40;
new claim 2 added; original claims 6-22 renumbered 7-23; remaining claims unchanged (9 pages)]

1. A water-soluble product or pharmaceutical
formulation in solid or liquid form and their organic solvent-
free true aqueous solutions mainly for parenteral use
containing
- a) a therapeutically active compound having low aqueous
solubility ($<1.10^{-4}$ M/Lit, $<1.10^{-5}$ M/Lit, $<1.10^{-6}$ M/Lit) and a
substantial binding affinity to plasma proteins (in the
following "active substance") in an interlinked state with
- b) a plasma protein fraction in controlled aggregation
state

- whereby the said active substance and the said protein fraction
are bound to each other by way of non-covalent bonds and
optionally further containing
- c) pharmaceutically acceptable and mainly parenterally
acceptable watersoluble formulation additive(s) - such as
water, stabilizer(s), protein aggregation controller(s).

2. A water-soluble product or pharmaceutical
formulation in solid or liquid form and their organic solvent-
free aqueous solutions according to claim 1 wherein the molar
ratio of the active ingredient : protein is within the range of
1 : 0.05 to 1 : 100, preferably 1 : 0.1 to 1 : 50

3. A water-soluble product or human pharmaceutical
formulation according to claim 1 or 2 containing a human plasma
protein fraction in controlled aggregation state.

4. A water-soluble product or veterinary pharmaceutical
formulation according to claim 1 or 2 containing an animal
plasma protein fraction in controlled aggregation state.

5. A product or pharmaceutical formulation according to
any of claims 1 to 4 containing as the plasma protein fraction
a component of the natural plasma such as serum albumin or a
recombinant of said plasma component.

6. A product or pharmaceutical formulation according to
any of claims 1 to 5 containing as the plasma protein fraction

a natural immunoglobulin, glycoprotein, interferon and/or interleukin or a recombinant of said plasma component.

7. A product or pharmaceutical formulation according to
5 any of claims 1 to 6 containing as the water-insoluble active substance a cytostatic such as a taxonoid, an antibiotic, vitamin, antiinflammatory, analgesic, antiviral, anticonvulsant, immunosuppressant, antiepileptic, anxiolytic, hypnotic, antifungal agent, anticoagulant, lipid peroxidase
10 inhibitor, coronary vasodilator, antiarrhythmic agent, cardio- tonic, uricosuric, antithrombotic, steroid hormone (progesterone, androgen, testosterone) and/or photosensitizer.

8. A product or pharmaceutical formulation according
15 to any of claims 1 to 6 containing at least one of the following active substances: amphotericin B, an adriamycin analogue, apazone, azathioprine, bromazepam, camptothecin, carbamazepine, clonazepam, cyclosporine A, diazepam, dicumarol, digitoxine, dipyridamole, disopyramide,
20 flunitrazepam, gemfibrozil, ketochlorin, ketoconazole, miconazole, niflumic acid, oxazepam, phenobarbital, phenytoin, progesterone, propofol, ritonavir, sulfinpyrazone, suprofen, tacrolimus, tamoxifen, taxonoid, testosterone, tirilazad, trioxsalen, valproic acid, warfarin.

9. A product or pharmaceutical formulation according
25 to any of claims 1 to 7 containing a taxonoid of the general formula I - in the formula

30 R^1 represents tert. butyl-oxy-carboxylic acid amide or benzoyl amide,

R^2 represents hydrogen or an acyl group preferably acetyl.

10. A product or pharmaceutical formulation according to
35 any of claims 1 to 7 containing paclitaxel and human serum albumin, immunoglobulin, glycoprotein, interferon and/or interleukin or some other natural or recombinant human plasma protein fraction in the molar ratio of 1 : 0.05 to 1 : 100, preferably in the molar ratio of 1 : 0.1 to 1 : 50.

40 11. A product or pharmaceutical formulation according to any of claims 1 to 9 containing azathioprine and human serum

albumin, immunoglobulin, glycoprotein, interferon and/or interleukin or some other natural or recombinant human plasma protein fraction in the molar ratio of 1 : 0.05 to 1 : 100, preferably 1 : 0.1 to 1 : 50.

5
12. A formulation according to any of claims 1 to 8 containing camptothecin and human serum albumin, immunoglobulin, glycoprotein, interferon and/or interleukin or some other natural or recombinant human plasma protein fraction
10 in the molar ratio of 1 : 0.05 to 1 : 100, preferably 1 : 0.1 to 1 : 50.

13. A formulation according to any of claims 1 to 8 containing gemfibrozil and human serum albumin, immunoglobulin,
15 glycoprotein, interferon and/or interleukin or some other natural or recombinant human plasma protein fraction in the molar ratio of 1 : 0.05 to 1 : 100, preferably 1 : 0.1 to 1 : 50.

20 14. A product or pharmaceutical formulation according to any of claims 1 to 8 containing miconazole and human serum albumin, immunoglobulin, glycoprotein, interferon and/or interleukin or some other natural or recombinant human plasma protein fraction in the molar ratio of 1 : 0.05 to 1 : 100,
25 preferably 1 : 0.1 to 1 : 50.

15. A product or pharmaceutical formulation according to any of claims 1 to 8 containing propofol and human serum albumin, immunoglobulin, glycoprotein, interferon and/or
30 interleukin or some other natural or recombinant human plasma protein fraction in the molar ratio of 1 : 0.05 to 1 : 100, preferably 1 : 0.1 to 1 : 50.

35 16. A product or pharmaceutical formulation according to any of claims 1 to 8 containing tamoxifen and human serum albumin, immunoglobulin, glycoprotein, interferon and/or interleukin or some other natural or recombinant human plasma protein fraction in the molar ratio of 1 : 0.05 to 1 : 100,
preferably 1 : 0.1 to 1 : 50.

40 17. A product or pharmaceutical formulation according to any of claims 1 to 8 containing ritonavir and human serum

albumin, immunoglobulin, glycoprotein, interferon and/or interleukin or some other natural or recombinant human plasma protein fraction the molar ratio of 1 : 0.05 to 1 : 100, preferably 1 : 0.1 to 1 : 50.

5

18. A pharmaceutical formulation according to any of claims 1 to 8 containing tacrolimus and human serum albumin, immunoglobulin, glycoprotein, interferon and/or interleukin or some other natural or recombinant human plasma protein fraction in the molar ratio of 1 : 0.05 to 1 : 100, preferably 1 : 0.1 to 1 : 50.

10

19. A pharmaceutical formulation according to any of claims 1 to 8 containing tirilazad and human serum albumin, immunoglobulin, glycoprotein, interferon and/or interleukin or some other natural or recombinant human plasma protein fraction in the molar ratio of 1 : 0.05 to 1 : 100, preferably 1 : 0.1 to 1 : 50.

15

20. A pharmaceutical formulation according to any of claims 1 to 8 containing trioxsalen and human serum albumin, immunoglobulin, glycoprotein, interferon and/or interleukin or some other natural or recombinant human plasma protein fraction in the molar ratio of 1 : 0.05 to 1 : 100, preferably 1 : 0.1 to 1 : 50.

20

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21. A pharmaceutical formulation according to any of claims 1 to 20 having a solid state or having the form of an aqueous solution.

30

22. A pharmaceutical formulation according to any of claims 1 to 20 containing as additive an agent stabilizing the solution and/or the protein.

35

23. A pharmaceutical formulation according to claim 22 containing as solution- and/or protein-stabilizing agent any of the following: sodium chloride, a buffer, an alcohol such as glycerol and/or a water-soluble sugar derivative preferably mannitol, sorbitol, dulcitol.

40

24. Process for the preparation of a water-soluble product or pharmaceutical formulation in solid or liquid form

according to any of claims 1 to 23 or a new product according to claims 30 to 37 and their organic solvent-free true aqueous solutions characterized by preparing a true aqueous solutions by way of

- 5 a) dissolving the therapeutically active compound having low aqueous solubility ($<1.10^{-4}$ M/Lit, $<1.10^{-5}$ M/Lit, $<1.10^{-6}$ M/Lit) and a substantial binding affinity to plasma proteins ("active substance") in a water-miscible, pharmaceutically acceptable organic solvent,
- 10 b) combining said solution with the aqueous solution of the plasma protein fraction in controlled aggregation state
- c) and optionally with a further pharmaceutically acceptable water-soluble auxiliary additive - such as a
- 15 protein aggregation controller and/or a stabilizer -

whereby a true solution is obtained containing the said active substance and the said protein fraction bound together by way of non-covalent bonds;

- 20 d) removing the organic solvent and optionally the water preferably by ultrafiltering, dialysing, diafiltrating and/or lyophilising the solution or its concentrate or by combination of these treatments

whereby a homogeneous, water-soluble liquid or solid

25 pharmaceutical product is obtained containing the active substance interlinked with the plasma protein fraction;

- e) optionally dissolving the solid product in water or diluting the liquid product with water whereby a clear, true aqueous solution, free of any organic solvent is
- 30 obtained which is suitable for therapeutical administration and

f) optionally finishing this product into a parenteral formulation (dosage form) for direct use.

- 35 25. Process for the preparation of a water-soluble product or pharmaceutical formulation in solid or liquid form according to any of claims 1 to 23 or a new product according to claims 30 to 37 and their organic solvent-free true aqueous solutions characterized by

- 40 a) dissolving the therapeutically active compound in a water-miscible, pharmaceutically acceptable organic solvent,

b) combining said solution with the aqueous solution of the selected plasma protein fraction in controlled aggregation state,

- c) said solution containing optionally a further
5 pharmaceutically acceptable auxiliary additive - such as a protein aggregation controller and/or a stabilizer -

whereby a true solution is obtained containing the said active substance and the said protein fraction bound together
10 by way of non-covalent bonds;

d) removing the organic solvent and lyophilising the solution or its concentrate.

26. A process according to step a) of any of claims 23
15 to 25 characterized by using to dissolve the active substance a solvent having the following properties:

- a) it is capable to completely dissolve the active substance in its mixture with water and
b) its mixture with <50% of water does not denaturalize
20 the protein employed.

27. A process according to claim 26 characterized by using as the solvent any of the group consisting of an aliphatic C(2-4) monoalcohol or polyalcohol,
25 70 - 100% ethanol, dimethyl formamide, methyl formamide.

28. A process according to step a) of claim 23 to 27 characterized by using as protein aggregation controller or stabilizer and/or solution stabilizing auxiliary
30 additive any of the following agents: water, sodium chloride, a buffer, a poly- alcohol such as glycerol and/or a water-soluble sugar derivative preferably mannitol, sorbitol and/or dulcitol.

29. A process according to step a) of any of claims 23
35 to 28 characterized by using paclitaxel and a component of the natural plasma such as serum albumin, an immunoglobulin, glycoprotein, interferon and/or interleukin or a recombinant of the same.

30. A homogeneous, solid, water-soluble product
40 consisting of at least one active substance having low aqueous

solubility ($<1.10^{-4}$ M/Lit, $<1.10^{-5}$ M/Lit, $<1.10^{-6}$ M/Lit) of the group amphotericin B, an adriamycin analogue, apazone, azathioprine, bromazepam, camptothecin, carbamazepine, clonazepam, cyclosporine A, diazepam, dicumarol, digitoxine, 5 dipyridamole, disopyramide, flunitrazepam, gemfibrozil, ketochlorin, ketoconazole, miconazole, niflumic acid, oxazepam, phenobarbital, phenytoin, progesterone, propofol, ritonavir, sulfinpyrazone, suprofen, tacrolimus, tamoxifen, taxonoid, testosterone, tirilazad, trioxsalen, valproic acid and/or 10 warfarin and also consisting of at least one protein of the group human serum albumin, immunoglobulin, glycoprotein, interferon and/or interleukin or some other natural or recombinant human plasma protein fraction 15 where the said active substance and the said protein fraction are bound to each other by way of non-covalent bonds and wherein the molar ratio of the said active substance and the said protein fraction is within the range of 1 : 0.05 to 1 : 100, preferably of 1 : 0.1 to 1 : 50.

20 31. A homogeneous, solid, water-soluble product according to claim 30 consisting of a taxonoid of the general formula I - in the formula
R¹ represents tert. butyl-oxy-carboxylic acid amide or 25 benzoyl amide,
R² represents hydrogen or any acyl group preferably acetyl - and of a plasma protein fraction.

30 32. A homogeneous, solid, water-soluble product according to claim 30 consisting of paclitaxel and human serum albumin, recombinant human plasma albumin and/or γ -globulin.

35 33. A homogeneous, solid, water-soluble product according to claim 30 consisting of amphotericin B and human serum albumin, recombinant human plasma albumin and/or γ -globulin.

40 34. A homogeneous, solid, water-soluble product according to claim 30 consisting of camptothecin and human serum albumin, recombinant human plasma albumin and/or γ -

globulin.

35. A homogeneous, solid, water-soluble product according to claim 28 consisting of carbamazepin and human serum albumin, recombinant human plasma albumin and/or γ -globulin.

36. A homogeneous, solid, water-soluble product according to claim 28 consisting of cyclosporin A and human serum albumin, recombinant human plasma albumin and/or γ -globulin.

37. A homogeneous, solid, water-soluble product according to claim 30 consisting of propofol and human serum albumin, recombinant human plasma albumin and/or γ -globulin.

38. Method of treatment of human or veterinary patients with a therapeutically active substance having low aqueous solubility ($<1.10^{-4}$ M/Lit, $<1.10^{-5}$ M/Lit, $<1.10^{-6}$ M/Lit) and having substantial plasma protein affinity characterized by administering to a patient in need of a treatment with said active substance an effective dose of the product or pharmaceutical formulation according to or prepared according to any of claims 1 to 37.

39. Method of treatment of human or veterinary patients with a therapeutically active substance having low aqueous solubility ($<1.10^{-4}$ M/Lit, $<1.10^{-5}$ M/Lit, $<1.10^{-6}$ M/Lit) and having substantial plasma protein affinity characterized by parenterally administering to a patient in need of a treatment with said active substance an effective dose of the following products preferably using the following dose ranges respectively (calculated on the active substance): paclitaxel/albumin 70 - 280 mg/treatment; propofol/albumin 6 - 10 mg/kg/hour; camptothecin/albumin, gemfibrozil/albumin, cyclosporin A/ albumin 3 - 5 mg/kg/day; amphotericin B/ albumin up to 1.5 mg/kg/day, whereby the same dose ranges are used for compounds containing the recombinant proteins respectively.

40. Method for parenteral delivery in therapeutic use of pharmaceutically active ingredients with poor solubility

($<1.10^{-4}$ M/Lit, $<1.10^{-5}$ M/Lit, $<1.10^{-6}$ M/Lit) and substantial affinity for binding to plasma proteins characterized by administering to a patient in need of a treatment with said active substance an effective dose of the composition
5 according to or prepared according to any of claims 1 to 29.

41. A product or method substantially as described in any of the examples.

STATEMENT UNDER ARTICLE 19

5

1. Original Claims 8 to 23, 29, 31, 32, 34, 36, 37, 39 to 41 were found to be new by the Search Report. These claims - with the exception of claim 40 - remained unchanged but to the correction of reference numbers where adequate. (They had to be partly renumbered because a new Claim 2 was inserted.)
2. Amended composition claim 1, new claim 2, amended process claim 24, compound claim 30, and method of treatment claims 39 to 40 are now amended by insertion of basically **three limitations:**

- a) It is now emphasized that pharmaceutical formulations to be used mainly for parenteral use **are organic solvent-free true aqueous solutions.**

This excludes pharmaceuticals for parenteral use which are

- i) suspensions or emulsions (such as those disclosed in EPA A1 326618 prepared by way of high speed stirring of a two or three phase system);
- ii) solutions and emulsions containing organic solvents such as ethanol (such as those disclosed in DE A1 3702105);

- b) The low aqueous solubility is now indicated to be $<1.10^{-4}$ M/Lit, $<1.10^{-5}$ M/Lit, $<1.10^{-6}$ M/Lit.
- This limitation excludes the use of active ingredients which have a higher aqueous solubility of about ≤ 0.1 mg/ml such as disclosed in the Patent Abstract of JP 58216126 mentioned in the Search report. Some of

such more soluble active ingredients were disclosed to form aqueous solutions by simple dissolution in water - which solutions in turn could be solubilized by addition of HSA as solubilizing agent.

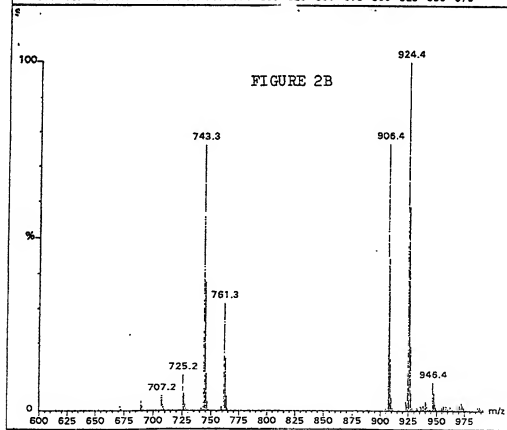
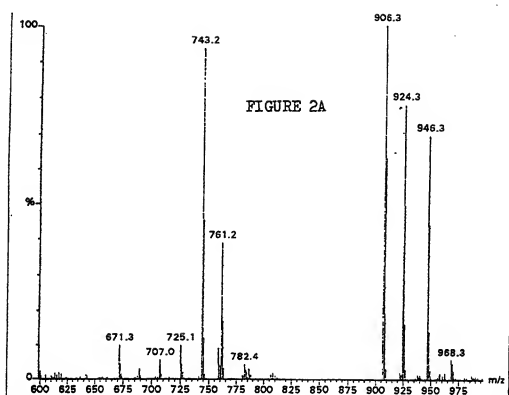
5 According to the present invention compounds having solubilities several magnitudes less can be transformed into water-soluble products - while the method disclosed in the mentioned prior art can not be used for products having solubilities as now claimed.

10 c) The limitation: *wherein the molar ratio of the active ingredient : protein is within the range of 1 : 0.05 to 1 : 100, preferably 1 : 0.1 to 1 : 50 .*

15 Claim 2 as well as all claims which are dependent therefrom exclude any ratios where the protein was present in a different ratio; such as e.g. WO 98/28011 published in the priority interval. The method disclosed there was related to one single antibiotic to be used in combination with a base (e.g. N-methyl-glucamine) and HSA in a molar ratio of 1 :
20 about 2-6 : about 0.006 to 0.030.

3. All limitations are based on the statements and facts disclosed in the specification and examples.
- 25 4. All claims now on file are considered to be **novel over** all citations contained in the Search Report.

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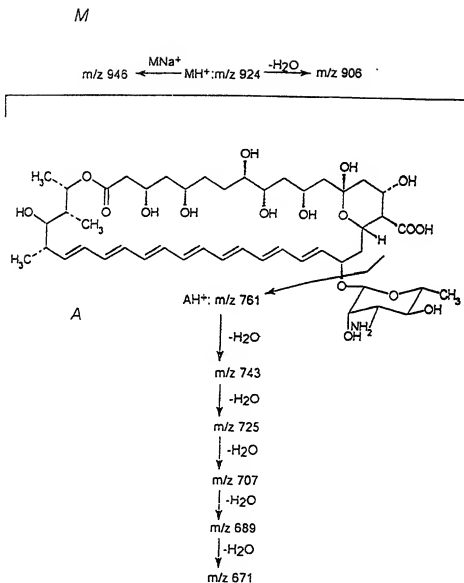
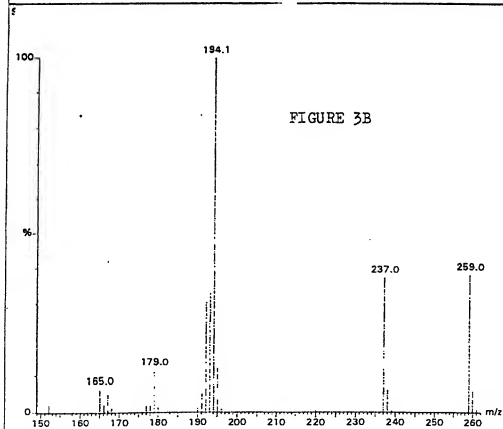
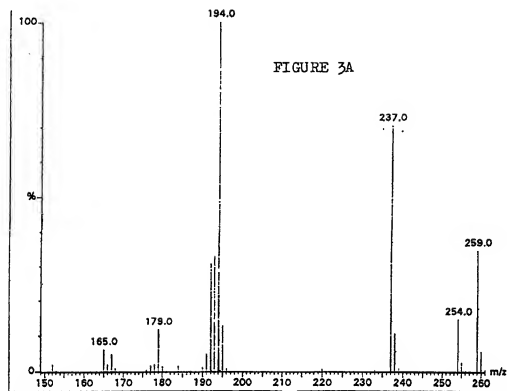


FIGURE 2C

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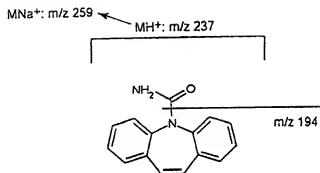
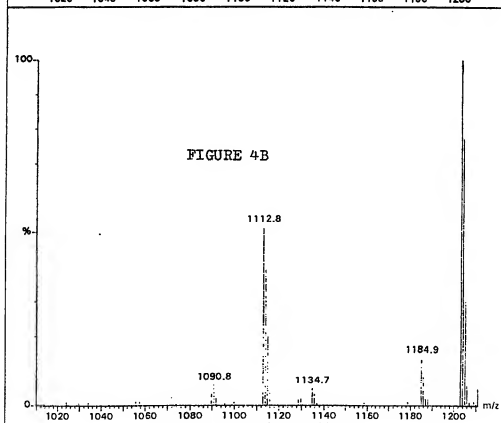
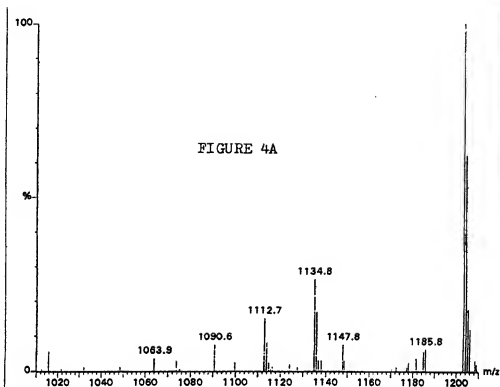


FIGURE 3C

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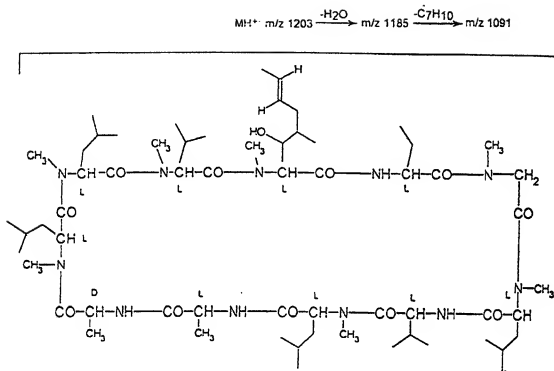
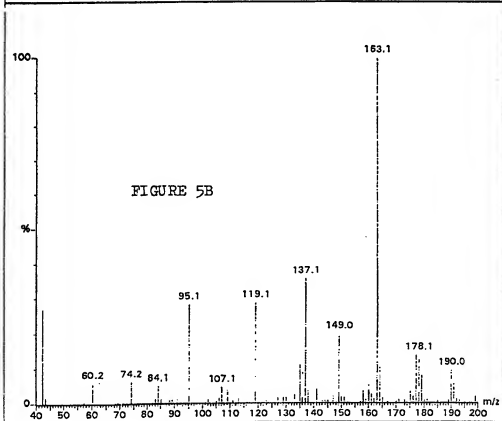
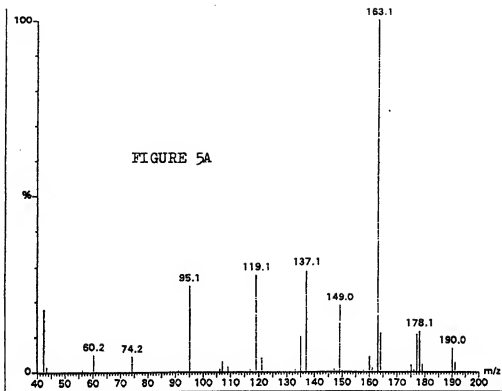
M series

FIGURE 4C

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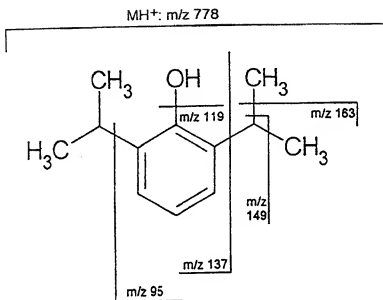
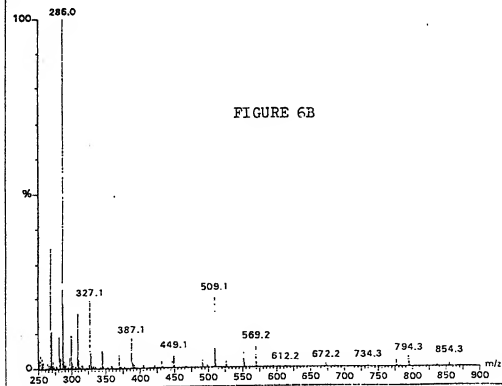
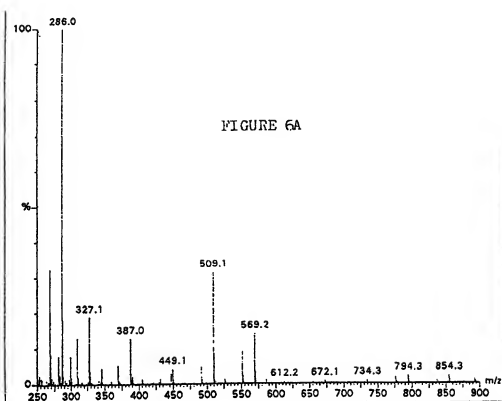


FIGURE 5C

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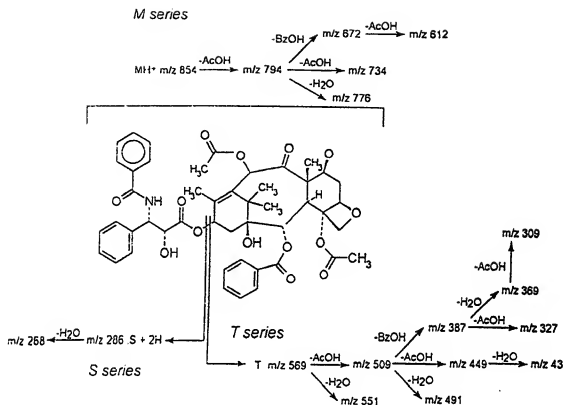


FIGURE 6C

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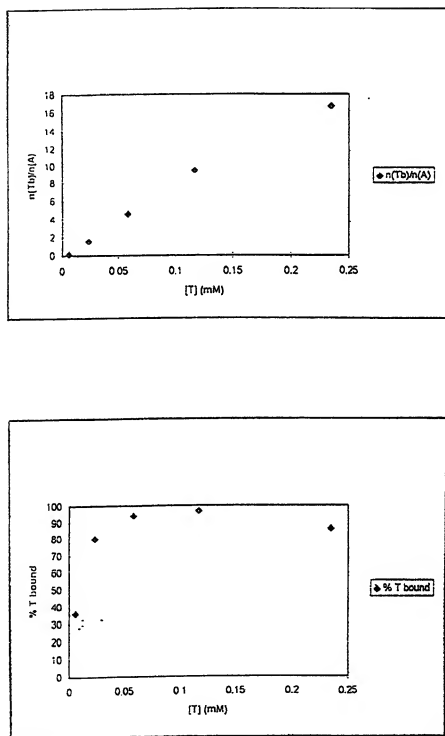


FIGURE 7

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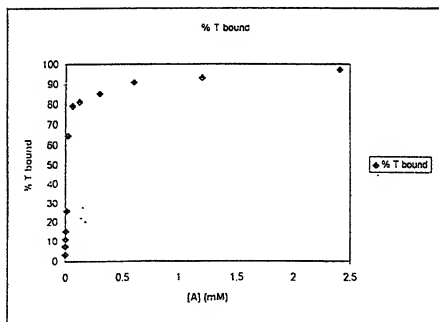
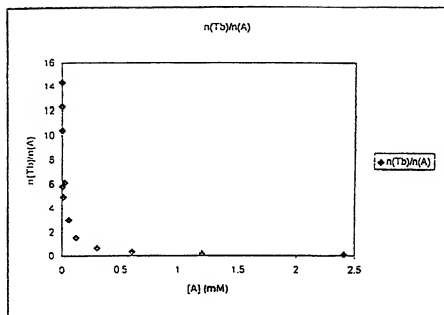
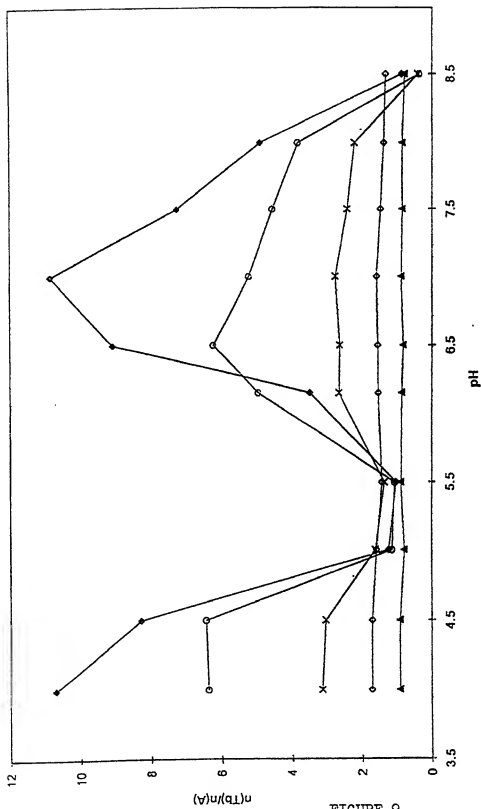


FIGURE 8

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INTERNATIONAL SEARCH REPORT

national Application No

PCT/HU 98/00086

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K47/42 A61K9/08

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category * Citation of document, with indication, where appropriate, of the relevant passages

Relevant to claim No.

X EP 0 326 618 A (NIPPON HYPOX LAB INC)
9 August 19891,2,4-7,
23-28,
30,33,
35,38see page 3, line 18-28
see page 5, line 12-17
see page 6, line 1
see page 7, line 3
see page 9, line 13-25
see claims 1,4X PATENT ABSTRACTS OF JAPAN
vol. 008, no. 062 (C-215), 23 March 1984
& JP 58 216126 A (ONO YAKUHI KOGYO KK),
15 December 1983
see abstract

1,2,4-7

-/-

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"I" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"Z" document member of the same patent family

Date of the actual completion of the international search

5 November 1998

Date of mailing of the international search report

11/11/1998

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
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Fax: (+31-70) 340-3016

Authorized officer

La Gaetana, R

INTERNATIONAL SEARCH REPORT

National Application No
PCT/HU 98/00086

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 37 02 105 A (BAYER AG) 4 August 1988 cited in the application see claims see page 2, line 64 - page 3, line 7 -----	1,2,4
P,X	WO 98 28011 A (SCHERING CORP) 2 July 1998 see page 2, line 21-32 see page 6, line 5-10 see page 6, line 19-21 see page 6, line 37-39 see page 25, line 9-22 see example 7 see claim 1 -----	1-4
P,A	WO 98 14174 A (SOON SHIONG PATRICK ;TAO CHUNLIN (US); YANG ANDREW (US); DESAI NEI) 9 April 1998 see page 11, line 22 - page 12, line 5 see page 12, line 15-25 see page 14, line 9-23 see page 17, line 13 - page 18, line 34 see page 22, line 1 - page 28, line 37 see claims 1-7 -----	1-41

INTERNATIONAL SEARCH REPORT

International application No.

PCT/HU 98/00086

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 38-40
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 38-40
are directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

national Application No
PCT/HU 98/00086

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
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